

results in repression of expression of a gene which is operatively linked to said DNA sequence until a temperature is reached that is 3 to 10° C higher than the temperature at which the wild type sequence is capable of repressing the expression of a gene operatively linked thereto, comprising

- f-1  
Gm'd
- (a) preparing a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one OR or OL operator DNA sequence from a lambdoid phage and a promoter,
  - (b) intentionally subjecting the operator DNA sequence to a mutagenesis, and
  - (c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.

46. An OR or OL operator sequence from lambdoid phages which have an increased thermostability compared to a wild-type sequence with regard to binding of a temperature-sensitive  $\text{cl}$  repressor, wherein said increased thermostability results in repression of expression of a gene which is operatively linked to said DNA sequence until a temperature is reached that is 3 to 10° C higher than the temperature at which the wild type sequence is capable of repressing the expression of a gene operatively linked thereto, and wherein said sequences are obtained by a method comprising

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- (a) preparing a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one OR or OL operator DNA sequence from a lambdoid phage and a promoter,
- (b) intentionally subjecting the operator DNA sequence to a mutagenesis, and
- (c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.
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f 3 69. A bacterial cell comprising at least one copy of a nucleic acid, wherein said nucleic acid comprises (a) a first bacterial expression control sequence which contains an OR or OL operator sequence from a lambdoid phage and to which a first  $\text{cl}$  repressor from lambdoid phages can bind, in operative linkage with a sequence coding for a second repressor wherein the second repressor cannot bind to the first bacterial expression sequence and (b) a second bacterial expression control sequence to which the second repressor can bind in operative linkage with a suicide gene, wherein said first bacterial expression control sequence is an operator sequence from a lambdoid phage wherein said sequence has a different thermostability compared to a wild-type sequence with regard to binding of a repressor wherein said different thermostability results in repression of expression of a gene which is operatively linked to said DNA sequence until a temperature is reached that is 3 to 10° C higher than the temperature at which the wild type sequence is capable of repressing the expression of a gene operatively linked thereto, and wherein said operator sequence is obtained by a method comprising

- F<sub>3</sub> cont'd
- (a) preparing a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one OR or OL operator DNA sequence from a lambdoid phage and a promoter,
  - (b) intentionally subjecting the operator DNA sequence to a mutagenesis, and
  - (c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.

70. A bacterial cell comprising at least one copy of a nucleic acid, wherein said nucleic acid comprises (a) a first bacterial expression control sequence which contains an OR or OL operator sequence from a lambdoid phage and to which a first  $\text{cl}$  repressor from lambdoid phages can bind, in operative linkage with a sequence coding for a second repressor wherein the second repressor cannot bind to the first bacterial expression sequence and (b) a second bacterial expression control to which the second repressor can bind in operative linkage with a suicide gene, further comprising (c) a third bacterial expression control sequence which contains a operator sequence in operative linkage with a suicide gene, wherein said operator sequence is from a lambdoid phage and wherein said operator sequence has a different thermostability compared to a wild-type sequence with regard to binding of a repressor, wherein said different thermostability results in repression of expression of a gene which is operatively linked to said DNA sequence until a temperature is reached that is 3 to 10° C higher than the temperature at which the wild type sequence is capable of repressing the expression of a gene operatively linked thereto, and wherein said operator sequence is obtained by a method comprising

- f<sub>3</sub> cont'd
- (a) preparing a DNA cassette which contains a selection gene under the operative control of an expression control sequence comprising at least one OR or OL operator DNA sequence from a lambdoid phage and a promoter,
  - (b) intentionally subjecting the operator DNA sequence to a mutagenesis, and
  - (c) analyzing the operator DNA sequences to determine whether said sequences have a different thermostability as compared to a wild-type sequence with regard to binding a repressor.

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Please add new Claims 77-78 and cancel Claims 47, 66, 67, and 68 without prejudice.

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--77 (new) The bacterial cell of claim 69, wherein said bacterial cell further comprises a gene for a first  $\text{cl}$  repressor.

fu 78. (new) The bacterial cell of claim 70, wherein said bacterial cell further comprises a gene for a first  $\text{cl}$  repressor. --

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#### REMARKS

Claims 38-76 are pending in this application. By this Amendment, Claims 38, 46, 69, and 70 have been amended. Claims 47, 66, 67, and 68 have been cancelled without prejudice and new Claims 77 and 78 have been added. Claim 49 stands allowed. Claims 38-48 and 50-76 have been rejected.

Applicant thanks the Examiner and his supervisor for the courtesies shown to him and his representatives during the personal interview of October 22, 2002. As required by the Interview Summary Report, the following is a summary of the substance of the discussion conducted during that interview.